THE EFFECT OF ELEVATED TEMPERATURE ON COPPER TOXICITY TO THE THERMOPHILIC ALGA *SYNECHOCOCCUS LIVIDUS* (CYANOPHYCEAE)

L. E. Riley[1] and M. L. Ostrofsky[1]

Abstract.— The hypothesis that temperature influences the toxicity of copper to thermophilic Cyanophyceae was tested in a laboratory study with *Synechococcus lividus*. This thermophile was tested at copper concentrations from 0 to 200 ug/l, and temperatures from 40.0 to 50.0 C. It was found that an interaction between increased copper and temperature significantly decreased the rate of carbon assimilation, chlorophyll content, and photosynthetic efficiency.

Geothermal springs represent a remarkably unique and stable environment with respect to a large number of physical and chemical parameters. The temperatures of these springs rarely vary more than 2 C throughout the seasons. A constant flow rate with laminar flow characteristics exists which, among other things, minimizes the forces of erosion. Light intensity is high. The area around hot springs is usually devoid of trees and the water column is shallow. Nutrient replenishment is continuous in the flowing water system, so that nutrient deficiencies probably do not develop (Brock 1970).

Even in these seemingly ideal conditions, only a restricted flora exists. Due to the elevated temperatures of the thermal spring environment—approximately 50 C to well above the boiling point in fumaroles—prokaryotes are usually the sole inhabitants (Brock 1967a). The Cyanophyceae present have an upper temperature limit of 73–75 C. These algae are not merely subsisting, but are actually growing and thriving at a given location (Brock 1967b). This heat tolerance seems to be due to a number of factors, including the thermal stability of their photosynthetic membrane systems, the low Q_{10} value of respiratory rates preventing acceleration to lethal catabolism, the heat stability of the algal protoplasmic structures and the capacity of their proteins to endure high temperatures without denaturation, and the lack of competition in the environment (Brock 1974, Lewin 1962). Luxuriant growth is to be expected in these locations.

However, visible degradation of the algal mats has occurred in many thermal springs of Yellowstone National Park. This deterioration is particularly noticeable in those areas which are heavily frequented by visitors.

Changes in water temperature, nutrient concentration, flow rate, etc., may be eliminated as possible mechanisms for degradation due to the stability of the environment. An external factor exists as the remaining possibility—i.e., the introduction of copper coinage to the thermal springs (R. A. Hutchinson, Yellowstone National Park Geologist, pers. comm.).

Copper has long been widely used as an algicide. The recommended dose for algal control in alkaline water ranges from 0.2 to 2.0 mg/l (Trainor 1978), but can be as low as 50 µg/l for *Chlorella* (Bartlett et al. 1974). The chemical analysis of some of the major thermal springs of Yellowstone National Park indicates the copper concentration ranges from 1 to 9 µg/l (Brock, 1978), which appears to be much too low for the demonstrated algicidal effects. However, this does not preclude the possibility of increased toxicity at the elevated temperatures found in the thermal springs. These temperatures approach the critical maximum for life itself.

The possibility, therefore, exists that even low copper concentration in a thermal environment produce a detrimental effect on

[1]Biology Department, Allegheny College, Meadville, Pennsylvania 16335.

algal mats—i.e., an interactive effect exists between copper and heat.

METHODS

Axenic cultures of *Synecococcus lividus* (R. Castenholz, Department of Biology, University of Oregon, pers. comm.) were maintained in a general growth medium (Miller et al. 1978) with a 12-hour light:dark cycle. Cultures were frequently diluted to maintain cells in exponential growth phase.

Batch cultures were acclimated to a temperature (±0.01 C) for one week prior to each experiment. Following this acclimation period the culture volume was subdivided, and to each aliquot an amount of copper was added as $CuSO_4 \cdot 5H_2O$. Final concentrations were 0, 50, 100, 150, and 200 μg Cu/l. Aliquots were then incubated a further 24 hours after which each treatment was dispensed into triplicate 125 ml glass bottles for measurement of carbon assimilation, and an amount was filtered for chlorophyll analysis.

To each of the replicate bottles, we added 5 u Ci of 14-C bicarbonate (New England Nuclear). Cells were incubated for three hours, then membrane filtered and washed with distilled water. Filters were dried, placed in omnifluor, and activity measured by liquid scintillation. Because each treatment was handled in the same manner, radioactive counts per minute (CPM) were directly comparable among treatments. Chlorophyll-a was estimated from the optical density of ethanol extracts.

TABLE 1. Relative carbon assimilation (counts per minute) at experimental temperatures and copper concentrations.

	μg/l Cu				
Degrees C	0	50	100	150	200
	31409	8339	8595	7505	2922
	47990	10980	8027	4508	1259
40.0	49364	9436	7765	4059	1032
	46400	11315	11415	3382	2662
	44649	10932	9174	2421	1284
	44459	9157	11700	4087	1221
	28340	26293	9845	2294	1076
	31502	16756	8746	2468	631
42.5	39546	21045	7263	1845	463
	45936	13467	8034	2932	1399
	51584	12640	6336	1317	2477
	46953	12162	7490	3420	352
	218047	131760	53929	26266	8420
	195315	138318	53482	24028	6977
45.0	165850	126580	46425	24480	4832
	184091	113685	39251	23552	2999
	178200	131783	29053	21473	2426
	147796	137110	38914	20091	1499
	126228	46272	16153	4162	1033
	140663	29294	13862	2798	903
47.5	155604	26466	10830	2287	1459
	164761	22688	11420	1932	619
	155644	26186	11849	1257	580
	147319	22365	10548	2040	520
	53933	37702	11616	4812	521
	54506	33803	11605	6573	1762
50.0	62672	24093	12541	1846	803
	73887	21548	9130	1942	4422
	79563	17767	7518	2232	506
	62200	17893	12252	4387	826

This procedure was repeated at each of five growth temperatures: 40.0, 42.5, 45.0, 47.5, and 50.0 C. To facilitate statistical manipulation, all CPM and chlorophyll data were normalized with respect to the control treatment (0 μg/l Cu) to eliminate differences among treatments due to variations in starting population density.

Results and Discussion

Carbon assimilation data in terms of CPM are shown in Table 1. At all experimental temperatures increased copper concentration led to decreased carbon assimilation so that, at 200 μg, Cu/l assimilation was less than 5 percent of the control value. Using analysis of variance for two-way classification (Mendenhall et al. 1977) we found a significant interaction ($F_{16,125} = 3.31$, $p < 0.01$) between temperature and copper concentration.

Two possible causes for decreased carbon assimilation include decreased chlorophyll content per cell and depressed photosynthetic efficiency measured as carbon assimilation per unit chlorophyll. Table 2 shows the chlorophyll concentration of aliquot cultures after only 24 hours of incubation in the presence of copper. Again, at all experimental temperatures there is a significant decrease in chlorophyll with increased copper. There was also significant interaction ($F_{16,25} = 8.56$, $p < 0.01$) between temperature and copper.

Photosynthetic efficiency as measured by carbon assimilation per unit chlorophyll similarly decreased with increasing copper concentration (Table 3). Further, there was a significant interaction ($F_{16,125} = 12.44$, $p < 0.01$) between temperature and copper.

From these data it appears that copper interacting with temperature can cause significant depression of the photosynthetic activity of *Synechococcus lividus*. This appears to be caused by a decrease in chlorophyll content of the cell, and a lowered photosynthetic efficiency. It is possible that at temperatures higher than those examined only minute concentrations of copper may prove to be toxic to *S. lividus*. If this is so, and if our results may be extended to other thermophilic cyanophytes, this is a possible mechanism to

Table 2. Chlorophyll-a concentrations (μg/l) of aliquot cultures following 24-hour incubation with various copper concentrations.

	μg/l Cu				
Degrees C	0	50	100	150	200
40.0	52	42	41	37	31
	49	40	37	36	29
42.5	50	38	27	31	28
	47	36	34	31	28
45.0	98	80	64	75	58
	98	69	64	77	61
47.5	87	63	59	42	34
	90	62	66	41	33
50.0	85	55	48	37	27
	83	58	51	38	26

Table 3. Relative photosynthetic efficiency as CPM ^{14}C assimilated per mg chlorophyll. Control treatments were normalized to 100 percent.

	μg/l Cu				
Degrees C	0	50	100	150	200
40.0	100	28	28	14	6
42.5	100	55	31	9	5
45.0	100	94	37	16	4
47.5	100	27	12	3	2
50.0	100	59	28	12	7

explain the current deterioration of algal mats in many thermal springs.

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